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Influence of a Haemolymph Protein Fraction on the Binding of Juvenile Hormone in Homogenates of Insect Epidermis (*Plodia interpunctella* (Hübner))*

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Summary. A carrier protein fraction (CPF) from larval haemolymph was found to influence binding and catabolism of tritiated juvenile hormone (JH) in homogenates of larval epidermis. The CPF reduced binding of tritiated JH in all of the particulate fractions but did not alter the relative binding pattern when compared with JH alone. The CPF also protected the hormone from degradative enzymes in the membrane vesicle and microsomal + cytosol fractions but not in the nuclear and mitochondrial fractions. Preliminary evidence exists for high-affinity binding sites for JH in the nuclear and mitochondrial fractions. We conclude that the CPF influences catabolism of the tritiated JH but does not participate in subcellular recognition of JH in homogenized target tissue.

Juvenile hormone (JH), which is responsible for maintaining larval characteristics in insects until metamorphosis, is secreted into the haemolymph and transported to target tissues by proteins that selectively bind it. Lipoproteins and proteins of low molecular weight have been associated with the transport of the hormone (Trautmann, 1972; Whitmore and Gilbert, 1972; Emmerich and Hartmann, 1973; Kramer et al., 1974; Ferkovich et al., 1975). Moreover, carrier proteins of low mol. wt. isolated from *Manduca sexta* (Kramer et al., 1974) and *Plodia interpunctella* (Ferkovich et al., 1975) were found to protect JH against nonspecific esterases in the fourth and early fifth instars (Sanburg et al., 1975a, b and Kramer, personal communication). The carrier protein, in addition to this protective role in the haemolymph, may be involved in the interaction of JH with its target cells. In the present paper we report an examination of this latter possibility in *P. interpunctella*.

Materials and Methods

The JH carrier protein was isolated in a haemolyph protein fraction (CPF) from mid-fifth-instar larvae (12 mg/larva) by gel permeation chromatography and labeled with tritiated JH (*Hyalophora cecropia* C_{18}JH[7-ethyl-1,2-^{3}H(N)] 14.1 ci/mM, New England Nuclear Corp.) as described by Ferkovich et al. (1975). Epidermal homogenates were prepared from similarly

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aged larvae. Tritiated JH (8×10^{-8} MJH) alone and the tritiated-JH-CPF (1 mg protein) were each incubated with an aliquot of the epidermal homogenate (0.25 g wet wt./1.5 ml, 3.4 mg protein). The labeled homogenates were then fractionated on discontinuous sucrose gradients according to Ferkovich et al. (1974). JH was tentatively distinguished from its metabolites by thin-layer chromatography (Slade and Zibitt, 1972). A flow diagram of the fractionation scheme is presented in Figure 1.

The sucrose density gradient fractions were not homogeneous; however, the predominant organelles contained in each fraction were: F_1 and F_2, membrane vesicles; F_3, fragments of rough ER, small mitochondria; F_4, rough ER, large mitochondria, lysosomes; F_5, nuclear material, membrane fragments (Ferkovich et al., 1975). To facilitate discussion of JH binding in the particulate fractions the data obtained on each were combined and are reported as follows: MV, membrane vesicles (F_1+F_2); M, mitochondrial (F_3+F_4); and N, nuclear (F_5). The $20,000 \times g$ supernatant is referred to as M+S, the microsomal+supernatant fraction.

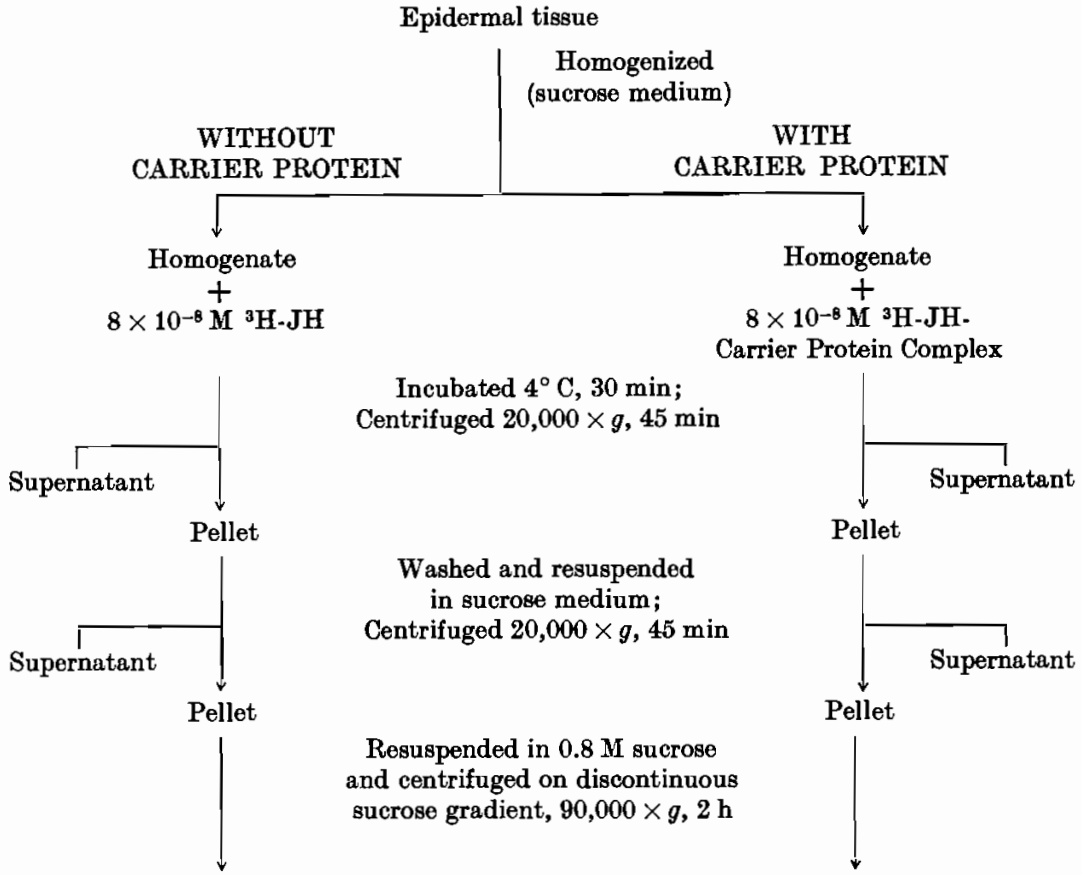
In another experiment, specific binding was determined according to King and Mainwaring (1974). Thus homogenized epidermis and ^{3}H-JH (3.36×10^{-8} M) were incubated at 4° C for 15 min. Then an unlabeled mixture of *H. cecropia* JH isomers (Hoffmann-La Roche) 3.36×10^{-4} M) was added to half of the homogenate, and both portions were incubated at 4° C for an additional 15 min. The same centrifugation procedure as described in Figure 1 was used, but in addition the $20,000 \times g$ supernatant was centrifuged at $100,000 \times g$ for 60 min to obtain the cytosol (soluble) and microsomal (pellet) fractions.

Results and Discussion

The specific radioactivity (dpm/50 μg protein) and relative amounts of unmetabolized ^{3}H-JH in fractions of homogenized epidermis incubated with and without the CPF is shown in Figure 2. The relative order of radioactivity/μg protein was: nuclear (N) < mitochondrial (M) < membrane vesicles (MV) < microsomal+supernatant fractions (M+S). The majority of the label recovered (>94%) remained in the $20,000 \times g$ supernatant that contained the microsomes and cytosol proteins. With the CPF, ^{3}H-JH binding was diminished in all the particulate fractions, probably because of the solubilizing effect of CPF on JH in the supernatant.

The percentage of bound ^{3}H-JH metabolized in the nuclear, mitochondrial, membrane vesicle, and microsomal+supernatant fractions was 44, 56, 75 and 85, respectively without the CPF; and 63, 66, 35 and 8, respectively with the CPF. Thus, in the presence of the CPF there was more degradation of ^{3}H-JH in the nuclear and mitochondrial fractions. In contrast, in the membrane vesicle and microsomal+supernatant fractions the hormone was protected.

The repeatability of the data is evident from the ratios of JH metabolism in the fractions in the presence/absence of the CPF. In one experiment, JH metabolism with/without freshly prepared carrier protein was: 2.32, 1.54, 0.43, and 0.15 for the nuclear, mitochondrial, membrane vesicle and microsomal+supernatant fractions, respectively. In another experiment, when we used reconstituted lyophilized protein that had been frozen and thawed several times and stored at −80° C for 6 months, the pattern of JH hydrolysis was similar: 1.43, 1.18, 0.47, and 0.09 for the respective fractions listed above. Our interpretation of the higher JH catabolism in the nuclear and mitochondrial fractions with the CPF is that the fractions contained high-affinity binding sites for JH (possibly receptors) which selectively removed JH from the protective influence of carrier protein. This, in turn, permitted the degradative enzymes access to the hormone. In contrast, the microsomal plus cytosol fractions may not have contained high-



Five particulate bands located at the surface of the gradient (0.8 M sucrose, F_1) and at each sucrose interface (0.8–1.0, 1.0–1.2, 1.2–1.5, 1.5–1.8), termed F_2 through F_5, respectively.

Fig. 1. Procedure for the centrifugation method used for subcellular fractionation of epidermal tissue incubated with ^{3}H-JH with and without the CPF

affinity binding sites, allowing the carrier protein to exert its protective influence. However, it is also possible that the nuclear and mitochondrial fractions contained enzymes comparable to the carrier bound JH esterases in the haemolymph of *Manduca sexta* (Sanburg et al., 1975a, b).

Further information concerning the specificity of binding in the fractions was sought by incubating ^{3}H-JH with a homogenate of epidermis with or without an excess of unlabeled JH. In essence, JH bound to low-affinity sites should be displaced by the unlabeled JH, whereas JH bound to high-affinity sites should not be displaced (King and Mainwaring, 1974).

The percent ^{3}H-JH bound/50 μg protein with excess JH added was 2.3, 9.9, 56.0, 10.9, and 20.7% in the nuclear, mitochondrial, membrane vesicle, microsomal, and supernatant fractions, respectively. Without the unlabeled JH, the percentage of ^{3}H-JH bound/50 μg protein in comparable fractions was 0.6, 3.2, 71.0, 4.1, and 21.1, respectively. The excess unlabeled JH reduced the percent ^{3}H-JH bound in the membrane vesicle fraction, indicating low-affinity binding. In contrast, the radioactivity increased in the nuclear, mitochondrial and microsomal fractions, suggesting the presence of high-affinity JH binding sites in these fractions. Apparently, binding of labeled JH to both low- and high-affinity

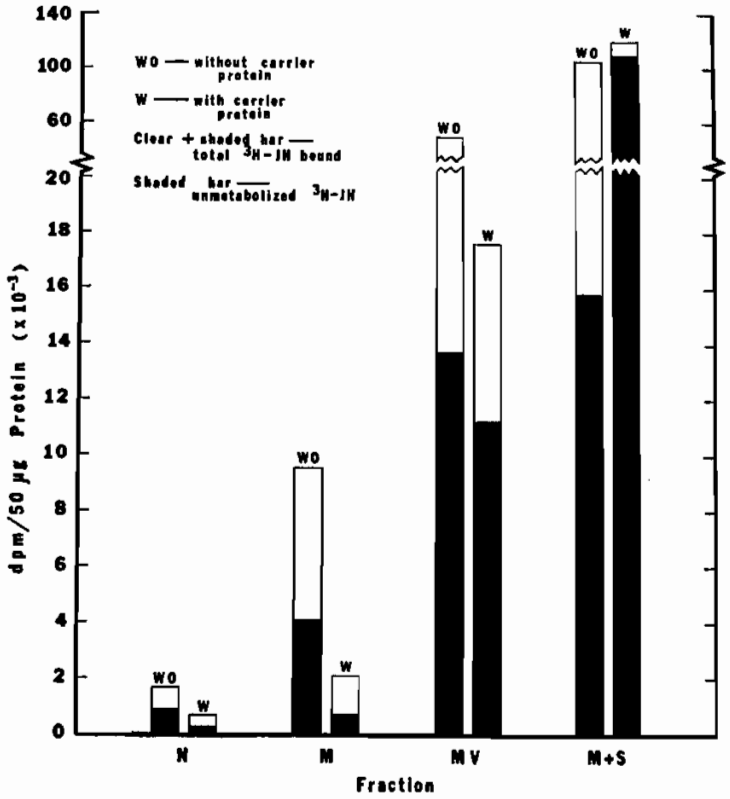


Fig. 2. Distribution of ^{3}H-JH (8×10^{-8} m) in subcellular fractions of epidermal homogenates incubated with and without CPF. *N* nuclear; *M* mitochondrial; *MV* membrane vesicle; and *M* + *S* microsomal + supernatant fraction

sites occurred initially; however, the amounts of JH bound decreased during the remainder of the 30-min incubation due to breakdown of the hormone. When excess unlabeled JH was added, it probably displaced labeled JH from the low-affinity sites and saturated the degradative enzymes. This resulted in a reduction in label in the membrane vesicle fraction having low-affinity sites and an increase in the nuclear, mitochondrial and microsomal fractions having high-affinity sites.

The primary step in JH action would then appear to be the interaction of the hormone with hypothetical specific receptors in the target tissue (Slàma et al., 1974). We find support for this hypothesis in the recent report by Schmialek (1973) of the isolation of a detergent-solubilized ribonucleoprotein receptor for a JH analogue in pupae of *Tenebrio molitor*. Our binding data indicated that the nuclear, mitochondrial, and perhaps the microsomal fractions contained high-affinity binding sites for JH. However, these results are preliminary, and additional criteria (such as varying concentrations of hormone, competitive binding by JH analogs, etc.) must be examined before one can firmly conclude that the fractions do indeed contain specific receptors for JH.

In the present study, we were investigating the possibility that the JH-carrier protein complex should bind only at receptor sites that either "recognize" the complex or that have a relatively greater affinity for JH than does the carrier

protein, thus effecting transfer of the hormone to the receptors. Based on the data obtained, we tentatively assume that the nuclear and mitochondrial fractions contain specific receptors for JH. Therefore, the JH-carrier protein complex probably is not necessary for recognition of the hormone at these sites. This is based on the observation that similar binding patterns were obtained in epidermal homogenates with JH alone or with the JH-carrier protein complex.

Finally, we found that epidermal target tissue contains enzymes which degrade JH and that the carrier protein influences such catabolism in vitro. Protection of JH by carrier protein in vitro was observed in fat body and imaginal wing disks of *Manduca sexta* (Hammock et al., 1975). Whether such protection also occurs in epidermal target tissue in *P. interpunctella* in vivo, however, is not known. In fact, we do not know whether the JH-carrier protein complex enters the cell once it arrives at the target tissue.

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